

AMENDMENT

U.S. Appln. No. 10/533,166 (Q87648)

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

Claim 1. (Withdrawn-Currently Amended) An assay method for detecting fungal infection of soil or vegetables by a pathogenic *Pythium* species, said method comprising:

- (A) treating a soil or vegetable sample to lyse fungal cells therein;
- (B) carrying out a polymerase chain reaction on DNA released by lysis of the fungal cells, using an oligonucleotide primer pair; and
- (C) detecting DNA fragments generated by said polymerase chain reaction so as to detect said fungal infection;

wherein said primer pair comprises an 18- to 24-mer which hybridizes under high stringency conditions to an oligonucleotide sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIIb (SEQ ID NO:12), VIIib (SEQ ID NO:14), VIIib (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20);

- 5' AGA CCA CAA TAA AGC GGC 3' — (Ib)
- 5' ACT TCT CTC TTT GGG GAG TGG 3' — (IIb)
- 5' TCG TTT CGG CTA TGA ATA CAG 3' — (IIIb)
- 5' ACA AAT ATA CCA ACC ACA GCG 3' — (IVa)
- 5' TTT GTA CTT GTG CAA TTG GC 3' — (IVb)
- 5' AAC GAA TAT ACC AAC CGC TG 3' — (Va)
- 5' TCA TCT ATT TGT GCA CTT CTT TTT 3' — (Vb)
- 5' GCC GCT TTA TTG TGG TCT 3' — (VIb)
- 5' CCA CTC CCC AAA GAG AGA AGT 3' — (VIIb)
- 5' CTG TAT TCA TAG CCG AAA CGA 3' — (VIIIb)
- 5' CGC TGT GGT TGG TAT ATT TGT 3' — (IXa)

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____ 5' GCC AAT TGC ACA AGT ACA AA 3' (IXb)
____ 5' CAG CGG TTG GTA TAT TCG TT 3' (Xa)
____ 5' AAA AAG AAG TGC ACA AAT AGA TGA 3' (Xb).

Claims 2-3. (Cancelled).

Claim 4. (Withdrawn-Currently Amended) The assay method as claimed in claim 1, wherein said primer pair comprises a pair of 18- to 24-mers which hybridize under high stringency conditions to a pair of the oligonucleotide sequences selected from the group consisting of formulae Ia (SEQ ID NO:1) and Ib (SEQ ID NO:2), IIa (SEQ ID NO:3) and IIb (SEQ ID NO:4), IIIa (SEQ ID NO:5) and IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7) and IVb (SEQ ID NO:8), and Va (SEQ ID NO:9) and Vb (SEQ ID NO:10)

____ 5' TCA CTT GTG GGG TAA AGA AGA 3' (Ia)
____ 5' AGT CCC GCA CAC ACA CAT 3' (IIa)
____ 5' TTC GTT CAG CCT CTG CAT 3' (IIIa).

Claim 5. (Cancelled).

Claim 6. (Withdrawn-Currently Amended) An assay method for detecting fungal infection of soil or vegetables by pathogenic *Pythium* species, said method comprising:

- (A) treating a soil or vegetable sample to lyse fungal cells therein;
- (B) carrying out a polymerase chain reaction on DNA released by lysis of the fungal cells, using an oligonucleotide primer pair;
- (C) contacting DNA fragments generated by said polymerase chain reaction with a substrate having immobilized thereon a primer which comprises an 18- to 24-mer which hybridizes under high stringency conditions to an oligonucleotide sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ

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ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20);

5' AGA CCA CAA TAA AGC GGC 3' (Ib)

5' ACT TCT CTC TTT GGG GAG TGG 3' (IIb)

5' TCG TTT CGG CTA TGA ATA CAG 3' (IIIb)

5' ACA AAT ATA CCA ACC ACA GCG 3' (IVa)

5' TTT GTA CTT GTG CAA TTG GC 3' (IVb)

5' AAC GAA TAT ACC AAC CGC TG 3' (Va)

5' TCA TCT ATT TGT GCA CTT CTT TTT 3' (Vb)

5' GCC GCT TTA TTG TGG TCT 3' (VIb)

5' CCA CTC CCC AAA GAG AGA AGT 3' (VIIb)

5' CTG TAT TCA TAG CCG AAA CGA 3' (VIIIb)

5' CGC TGT GGT TGG TAT ATT TGT 3' (IXa)

5' GCC AAT TGC ACA AGT ACA AA 3' (IXb)

5' CAG CGG TTG GTA TAT TCG TT 3' (Xa)

5' AAA AAG AAG TGC ACA AAT AGA TGA 3' (Xb); and

(D) detecting DNA fragments binding to said primer so as to detect said fungal infection.

Claim 7. (Currently Amended) An 18- to 24-mer oligonucleotide primer which hybridizes under high stringency conditions to an oligonucleotide sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20).

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Claims 8-9. (Cancelled).

Claim 10. (Currently Amended) The primer as claimed in claim 7, wherein said primer comprises a sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20).

Claim 11. (Currently Amended) A substrate having immobilized thereon at least one 18- to 24-mer oligonucleotide primer which hybridizes under high stringency conditions to an oligonucleotide sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20).

Claim 12. (Currently Amended) The substrate as claimed in claim 11, wherein said primer comprises a sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20).

Claim 13. (Currently Amended) A primer composition comprising a pair of 18- to 24-mer oligonucleotide primers, at least one of which hybridizes under high stringency conditions to an oligonucleotide sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID

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NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20), optionally together with a carrier.

Claim 14. (Currently Amended) The primer composition as claimed in claim 13, wherein at least one of said pair is a primer comprising a sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20).

Claims 15-16. (Cancelled).

Claim 17. (Currently amended) A kit for detecting fungal infection of soil or vegetables by a pathogenic *Pythium* species, said kit comprising at least one pair of 18- to 24-mer oligonucleotide primers, at least one of which hybridizes under high stringency conditions to an oligonucleotide sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20), together with instructions for the performance of the assay method.

Claims 18-21. (Cancelled).

Claim 22. (Currently amended) The kit as claimed in claim 17, wherein at least one of said pair is a primer comprising a sequence selected from the group consisting of formulae Ib (SEQ ID NO:2), IIb (SEQ ID NO:4), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20), optionally together with a carrier.

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NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIb (SEQ ID NO:12), VIIb (SEQ ID NO:14), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19, and Xb (SEQ ID NO:20).

Claim 23. (withdrawn-new) The assay method as claimed in claim 1, 4, or 6, wherein said high stringency conditions comprise washing two times with SSC (0.15M NaCl, 0.015M sodium citrate, pH 7.2) at 65 °C.

Claim 24. (new) The oligonucleotide primer as claimed in claim 7, wherein said high stringency conditions comprise washing two times with SSC (0.15M NaCl, 0.015M sodium citrate, pH 7.2) at 65 °C.

Claim 25. (new) The substrate as claimed in claim 11, wherein said high stringency conditions comprise washing two times with SSC (0.15M NaCl, 0.015M sodium citrate, pH 7.2) at 65 °C.

Claim 26. (new) The primer composition as claimed in claim 13, wherein said high stringency conditions comprise washing two times with SSC (0.15M NaCl, 0.015M sodium citrate, pH 7.2) at 65 °C.

Claim 27. (new) The kit as claimed in claim 17, wherein said high stringency conditions comprise washing two times with SSC (0.15M NaCl, 0.015M sodium citrate, pH 7.2) at 65 °C.